INFLUENCE OF pH ON FREEZE-THAW LETHALITY IN *STAPHYLOCOCCUS AUREUS*

PAUL H. DEMCHICK, SAMUEL A. PALUMBO and JAMES L. SMITH

Eastern Regional Research Center[1]
Philadelphia, Pennsylvania 19118

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ABSTRACT

The influence of pH on the susceptibility of Staphylococcus aureus *196E to repeated freeze-thaw stress was studied in 0.2 M acetate buffer (pH 3.0-7.8) and ground beef (adjusted to pH 4.2-6.3). In acetate buffer in the pH range of 4.4-7.0, repeated freeze-thaw stress did not decrease the viability of the cells; at pH values below 4.1 and above 7.5, decrease in the viable cells was exponential with the number of freeze-thaw cycles. In meat,* S. aureus *was not killed at pH values 4.3-6.3; however, at pH 4.2, death resulted from the repeated stress. Repeated freeze-thaw cycling of foods should have little effect on the viability of* S. aureus *within the pH values of most foods implicated in* S. aureus *food poisoning.*

INTRODUCTION

Microorganisms may be lethally stressed by freezing followed by thawing (Kraft and Ray 1979). Previous studies on the degree of injury or killing by freeze-thaw stress have considered the influence of initial cell count (Major *et al.* 1955), length of frozen storage time (Harrison 1955), rate of temperature change and actual temperatures studied (Mazur 1961), nature of the suspension fluid (Straka and Stokes 1959), moisture content (Mazur 1961; Schmidt-Lorenz 1976), and presence of low concentrations of chemical agents (Takano *et al.* 1979). However, in those studies, the effect of pH of the frozen-thawed suspension was not mentioned. Many researchers examining freeze-thaw stress on bacteria do not even report the pH of the stress media used (Straka and Stokes 1959; Postgate and Hunter 1963; Baird-Parker and Davenport

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1965; Takano *et al.* 1979). The objective of the present study was to determine the effect of pH on the viability of *Staphylococcus aureus* subjected to freeze-thaw cycling in meat and buffered broth systems.

MATERIALS AND METHODS

S. aureus 196E was inoculated into 100 ml of tryptic soy broth (Difco)[1] and incubated at 37° C on a rotary shaker (200 rpm) for 16 h. Cells were harvested by centrifugation (5 min, 16,000 × g, 5° C). The surface of the pellet was rinsed with distilled water.

For use in the liquid stress media, cells were resuspended in 100 ml of distilled water. Cells were diluted and placed in prechilled (5° C) acetate buffer (acetic acid, sodium acetate) so that the final cell concentration was approximately 10^5 per ml and the acetate ion concentration was 0.20 M. These procedures were performed as rapidly as possible, so that the cells were held in the chilled acetate buffer for less than 5 min. For use in the meat stress media, the cells were resuspended in 100 ml of 0.1% peptone (Difco) solution. Two ml of the cell suspension were mixed into 155 g of fresh ground beef (lean, chuck). Inoculated beef was divided into 4 portions and acetic acid (49%) solution was added to 3 of the portions to adjust the pH. From each of the 4 batches of inoculated meat, 5g portions were placed into tubes; tubes were held at 5° C until the meat had been acidified for 35 min.

After the initial holding period at 5° C, the viable count was determined. All broth and meat samples were then placed in a dry ice-ethanol bath for 5 or 7 min, respectively. The broth and meat tubes were removed from the dry ice-ethanol bath and thawed in a 20° C water bath for 10 or 15 min, respectively. After thawing, one set of tubes were held at 5° C while viable counts were determined (within 30 min after thawing), and the remainder of the tubes were subjected to additional freeze-thaw cycling. The freeze-thaw cycles were repeated four times.

Samples from the buffer system were diluted with 0.1% peptone solution, and surface-plated on tryptic soy agar (Difco; TSA). Meat samples were homogenized in 100 ml of peptone solution with a Stomacher 400 Lab Blender (Dynatech Laboratories, Alexandria, Virginia) and appropriate dilutions were plated on TSA. All plates were incubated at 37° C for 48 to 72 h. Only golden pigmented colonies were counted, with appropriate colonies being routinely checked for mor-

[1]Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

phology and gram reaction. The limit of detection was one viable cell/2 ml. The pH was measured with a Beckman Expanded Scale pH Meter (Model 76) using a combination pH electrode (I-0 2000, Owens-Illinois, Inc., Toledo, Ohio).

RESULTS AND DISCUSSION

The effect of pH on the survival of *S. aureus* subjected to freeze-thaw cycling in buffer and meat systems is summarized in Tables 1 and 2, respectively. In acetate buffer with pH values between 4.4 and 7.0, freeze-thaw cycling had no effect on the viability of *S. aureus*. However, at pH values 3.1 to 4.1 and 7.5 to 7.8, there was destruction of staphylococci during freeze-thaw stress (Table 1). When the meat samples were exposed to freezing and thawing, *S. aureus* was not killed when the pH was 4.6 to 6.3, but at pH 4.2, there was destruction of the organims (Table 2). In both the buffer and meat systems, the lethality associated with the pH extremes appeared to be first order with respect to the number of freeze-thaw cycles. Preliminary examination of the cultures for differential salt tolerance (Smith *et al.* 1982) indicated that repeated freeze-thaw cycling did not produce a significant degree of sublethal stress (injury).

Table 1. Effect of freeze-thaw cycles on population of *Staphylococcus aureus* in liquid media

	Number of Freeze-Thaw Cycles Completed				
	0	1	2	3	4
pH	$\log_{10}$(cells/ml)				
7.8	5.26	4.01	3.65	2.48	0.56
7.5	5.27	4.27	3.94	3.64	2.99
7.0	5.26	5.22	5.24	5.23	5.21
6.1	5.23	5.22	5.22	5.22	5.27
5.3	5.24	5.27	5.22	5.23	5.24
5.1	5.22	5.24	5.24	5.27	5.21
4.7	5.23	5.22	5.26	5.22	5.23
4.6	5.24	5.23	5.24	5.21	5.22
4.4	5.24	5.20	5.22	5.11	5.08
4.1	5.21	3.79	2.96	1.96	0.84
3.8	5.24	3.80	2.38	1.76	0.78
3.1	5.25	3.58	1.38	BDL[a]	BDL[a]

[a]BDL = Below Detectable Limit
Log_{10} [(Detectable Limit) · (ml/cell)] = −0.3

Table 2. Effect of freeze-thaw cycles on population of *Staphylococcus aureus* in meat media

	Number of Freeze-Thaw Cycles Completed				
	0	1	2	3	4
pH	$\log_{10}$(cells/ml)				
6.3	5.19	5.18	5.19	5.18	5.20
5.6	5.15	5.15	5.14	5.14	5.15
4.6	4.99	4.94	4.95	4.92	4.87
4.2	4.43	3.70	3.41	3.46	2.98

Exposure of *S. aureus* containing liquid media or meat samples to 1 h incubation at 5 or 20° C without freeze-thaw stress did not affect the number of viable cells, indicating that the destruction of staphylococci was due to a combined low pH and freeze-thaw stress. The conditions used in this study, i.e., fast freezing followed by fast thawing which approximated thawing at temperature abuse conditions, probably ensured survival of *S. aureus* at pH values above 4.5 (MacLeod and Calcott 1976).

Most foods linked to *S. aureus* food poisoning outbreaks (Centers for Disease Control 1976-81) have pH's within the 4.4 to 7.0 range (Banwart 1979). Even if *S. aureus* were not killed in foods undergoining freeze-thaw, it is probable that they would not produce enterotoxin at pH values below 5 (Scheusner and Harmon 1973). This would not be true if the acid food containing *S. aureus* was combined with another food, yielding a pH suitable for toxin production. Within the pH found in most frozen foods, repeated freeze-thaw stress would probably not lead to a substantial decrease in the numbers of contaminating *S. aureus*.

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